Public Health England

The PHE National Parasitology Reference Laboratory

USER MANUAL

Version 8.0
Revised November 2013

Parasitology User Manual - Version: 8.0. Index: PARA-MM-UserManual. Printed: 04-Dec-2013 14:31

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Section 1

INTRODUCTION - ABOUT THE DEPARTMENT OF CLINICAL PARASITOLOGY

SERVICES PROVIDED

The PHE National Parasitology Reference Laboratory services requests from all General practitioners, PHE and Medical Microbiology laboratories in the NHS. The Department has an international reputation and provides a parasitology service to Clinicians and Laboratories world wide.

The Department offers a wide range of investigations including diagnosis and identification of parasites in clinical material, diagnosis of human parasitic disease by immunological methods, culture and genomic detection from clinical material.

A twenty-four hour service for microscopic diagnosis of malaria is available. An advisory service on investigation of patients for parasitic disease, the appropriateness of tests, their timing and interpretation together with advice on treatment is also available. Individual tuition for technical, scientific and medical staff in faecal and blood parasitology can be provided by special arrangement.

The Department of Clinical Parasitology processes over 33,000 requests per annum.

The Department also houses the UKNEQAS (Parasitology) laboratory and the UK NEQAS-associated teaching facility

REMIT OF THE DEPARTMENT OF CLINICAL PARASITOLOGY

The remit of the Department of Clinical Parasitology is:

1) **To provide a comprehensive diagnostic, identification and advisory service on human parasites and the diseases they cause.**
2) **To develop, evaluate and advise on new parasite diagnostic techniques.**
3) **To produce epidemiological data for the PHE.**
4) **To liaise with other diagnostic and research parasitology laboratories in the UK and overseas, so that best practice is shared globally**

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Section 2

HOW TO USE THIS MANUAL

The manual is intended to assist you in making the best use of the services offered by the Department of Clinical Parasitology. The manual is divided into six sections. If you have difficulty finding information that you think should be here, please let us know, so that we can improve the manual. (email david.manser@uclh.nhs.uk)

The page numbers on which specific items appear are listed in the Table of Contents at the front of the manual.

Section 1 of the manual provides an overview of the Department of Clinical Parasitology.

Section 2 of the manual explains how to use the manual

Section 3 of the manual provides information about the Department of Clinical Parasitology, its staff and organisation and contact details.

Section 4 of the manual provides suggestions on how to use the Diagnostic and Advisory Service.

Section 5 provides a repertoire of services available from the Department of Clinical Parasitology.

Section 6 describes how results are normally sent to you and how they can be obtained if they are required urgently.

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Section 3

THE DEPARTMENT OF CLINICAL PARASITOLOGY – STAFF AND ORGANISATION

The Department of Clinical Parasitology is a laboratory within the Specialty of Microbiology, UCLH.

The Department of Clinical Parasitology is the PHE National Parasitology Reference Laboratory.

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STAFFING:

Senior staff:

Professor P L Chiodini - Consultant Parasitologist

Mr D W Manser - Laboratory Manager

Dr C Sutherland - Clinical Scientist Grade B

Specialist Registrar (on rotation) in Parasitology

BMS 8a: Ms P Lowe –Serology Section Head
Ms J Watson – Microscopy & PCR Section Head

Laboratory staff:

BMS band 7: Dr WJ Bligh
Mr MM Suvari
Dr S Boadi
Ms K Bowers
Mrs C Fagg
Mrs R. Kinson
Ms Z Hrydziuszko

MLA: Mr A Cenizal
Ms C. Halls

CONTACTING SENIOR STAFF

Professor. P.L. Chiodini	020 3447 5418
Mr. D. W. Manser	020 3447 5411
Specialist Registrar	020 3447 5809
On-call BMS	0845 155 5000 or 020 34567890 and bleep UCH 425

ENQUIRIES

For enquiries requesting information and/or advice regarding any item identified in the list of services offered by the Department of Clinical Parasitology, requests for information and/or advice regarding suitability of specimens, significance of results and treatment options.

Consultant Parasitologist 020 3447 5418

Specialist Registrar 020 3447 5809

For enquires requesting information regarding safe arrival of specimens, availability of tests, etc.

Laboratory Manager 020 3447 5411

Or appropriate section: Microscopy / PCR 020 3447 5414
Serology 020 3447 5413

HOURS OF BUSINESS

Information and advice is available from staff in the Department of Clinical Parasitology within normal working hours (0900 - 1700 Monday to Friday).

EMERGENCY ON-CALL SERVICE

A 24 hour, 7 day service is provided for urgent malaria diagnosis.

URGENT REQUESTS DURING NORMAL HOURS

Please telephone to say that an urgent sample is en route.

Contact 020 3447 5418.

A responsible person (and deputy), capable of accepting and transmitting the result(s), in the submitting organisation must be identified at this time. The results of urgent tests will be telephoned by a senior member of staff to the identified person (or deputy) in the submitting organisation as soon as the result is verified

FABRIC AND FACILITIES

The department is situated in purpose built laboratory accommodation commissioned in December 1998. There are 5 laboratories: Microscopy; Serology; Research; UK NEQAS and PCR areas.

Routine access to the laboratories is restricted to the laboratory staff, with controlled entry for visitors. Many of the specimens arrive by vacuum tube, minimising the need for non-laboratory staff to enter the department.

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Section 4

HOW TO USE THE DIAGNOSTIC AND ADVISORY SERVICE

TRANSPORT AND COLLECTION OF SPECIMENS

Specimens are received (by vacuum tube, post, DX, hospital van, taxi, or by courier) at the Specimen Reception area of the department. A regular van delivery / pickup of specimens between local centres is maintained by the UCLH Transport department.

If specimens are to be brought to the laboratory personally by medical or nursing staff they must be carried in an approved container for transport.

SAFETY

Current guidelines must be followed to avoid needle stick injuries or accidental exposure to blood and blood-contaminated body fluids of those persons taking, transporting and processing the samples.

Any accident should be reported at once to your immediate superior as urgent action may be required; please refer to your local Safety Policy/Infection Control guidelines.

Neither the request form nor the outside of the container should be contaminated with the sample.

Ensure that the container is correctly sealed. All specimens from human sources must be regarded as potentially infectious

PACKAGING OF SPECIMENS

Label all samples clearly with hospital number, name, and date of collection.

Location, consultant code/name, doctor's name, bleep/extension and test or tests required in addition to the patient details above should be put on the request form.

The sender must be an authorised person, not a member of the public; the recipient of the results must be a recognised laboratory or medical practitioner.

Specimens MUST be packaged according to Packing Instructions P650 and UN3373 requirements. See "Transport of infectious substances - best practice guidance for

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microbiology laboratories" available on the Department off Health website (www.dh.gov.uk)

The outside must be marked conspicuously with the following:
'BIOLOGICAL SUBSTANCE, CATEGORY B'

It is essential that such substances are properly packed and labelled and appropriate instruction and protection provided to the carrier(s).

The sender is responsible for ensuring the health and safety of any courier or taxi service that is used to transport samples to the Parasitology laboratory.

Hazardous specimens

Any specimens from known or suspected cases of hepatitis, tuberculosis, or HIV/AIDS must be clearly identified as a 'RISK OF INFECTION'.

Spillage of body fluids / leaking containers. - This may necessitate the rejection of the specimen. If this occurs, a member of the Department of Clinical Parasitology staff will inform a responsible person in the submitting organisation by telephone and advise that a request for a repeat sample be made.

REQUEST FORMS

Where possible, use a Parasitology request form personalised to your location. A personalised request form will have the code assigned to your laboratory or practice, this ensures speedy processing of the specimen and ensures the report is returned to the requesting address. If a laboratory would like a copy of the new parasitology request form please email the Laboratory Manager providing the laboratory address, responsible person (or deputy) to whom results are to be sent, telephone and fax numbers to: david.manser@uclh.nhs.uk

Request forms can be dispatched to you by prior arrangement. Use a separate form for each specimen type. Complete all sections of the form using a ball-point pen or ink. Mark clearly the name of the responsible person (or deputy) to whom results are to be sent.

Please give complete patient identification and relevant clinical details, including risk category and travel history. This information is needed to help determine which special precautions are required and which tests are to be done.

Processing times for different specimens vary according to clinical priority, as does the frequency of individual tests.

CLINICALLY IMPORTANT REQUESTS WILL BE GIVEN PRIORITY AND THE RESULTS TELEPHONED TO YOU BY A SENIOR MEMBER OF STAFF AT THE EARLIEST OPPORTUNITY.

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TYPES OF SPECIMENS

Confirmation of Parasitic infection can often be obtained directly following the analysis of a clinical specimen for the presence of the parasite. Indirect methods can also be used to test for evidence of a parasitic infection. Negative results do not necessarily exclude a diagnosis.

Faeces, blood and sera constitute the majority of samples received for analysis. Other samples include adhesive tape smears, urine, skin snips, biopsies, and whole organisms such as arthropods and worms for identification.

Please send separated serum rather than whole blood for serology requests (to prevent lysis of sample if delayed in post)

If you are uncertain of the type(s) of specimen(s) you should submit for analysis, telephone prior to sending the sample, in order that you can discuss the appropriateness of the specimen with a senior member of staff from the Department of Clinical Parasitology.

POSTAL ADDRESS

Send your specimens, together with an official request form or signed letter containing as much clinical information as is deemed necessary and requesting the service(s) required to:

The Department of Clinical Parasitology
The Hospital for Tropical Diseases
3rd Floor Mortimer Market Centre
Mortimer Market
London WC1E 6JB

Dx Number: DX 6640701
Exchange: TOTTENHAM CT RD 91 WC

Please Note: Specimens sent for diagnosis or further investigation to a clinical laboratory must comply with the conditions set down in the Post Office Regulations governing the transport of pathological specimens. For insurance purposes, the value of a routine specimen is not likely to exceed £1 sterling.

Specimens which are known or suspected to contain Hazard Group 4 pathogens should not be sent by post.

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Section 5

Repertoire of Services offered by the Department of Clinical Parasitology

The Department of Clinical Parasitology offers the following services:

1. Diagnosis and identification of parasites in clinical material, for example:

a) Identification or confirmation of identity of ova, cysts, larvae and worms in faeces, tissues, urine and other fluids. The department aims to provide a 24 hour turnaround time within the working week for the above-mentioned specimens. If histology is required the sample will be dealt with in conjunction with a histopathologist.

b) Speciation of malaria and filaria parasites in thick and thin blood films.
The department aims to provide a 24 hour turnaround time, within the working week, for these specimens. Communication with the laboratory before the specimen is dispatched is recommended for urgent samples.

2. Diagnosis of human parasitic diseases by immunological methods.

3. Culture of *Leishmania* from clinical material by prior arrangement.

Culture of *Leishmania* from clinical material can take up to THREE WEEKS. Prior arrangement is advised to obtain the most efficient service.

4. PCR assays

PCR assays for *Leishmania*, *Microsporidia* and the triple assay for *E. histolytica*, *Giardia* and *Cryptosporidia* are available on request.

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5. Advisory service

We provide an advisory service on the investigation of patients for parasitic disease, the appropriateness of tests, their timing and interpretation together with advice on treatment.

Information regarding this service can normally be provided by telephone, fax or email.

SPECIMENS REQUIRED FOR THE DIAGNOSIS OF INDIVIDUAL PARASITIC DISEASES

Amoebiasis *(Entamoeba histolytica)*

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Intestinal - stool samples for the detection of *Entamoeba histolytica / Entamoeba dispar* cysts can be sent by conventional means. Examination for trophozoites requires that the stool is examined within 30 minutes of voiding. Accordingly the laboratory must be advised in advance of submission. Microscopy of rectal scrapings must be arranged with the laboratory in advance.

The molecular test for *Cryptosporidium* species, *Giardia intestinalis* and *Entamoeba histolytica* offers several advantages over standard microscopy based diagnostics. The assay is significantly more sensitive (greater than ten fold improvement in the limit of detection for some species) than light microscopy. In addition the assay is semi quantitative and can therefore reveal detailed information on the response of a patient's parasite load to subsequent drug therapy. For *Entamoeba histolytica* the assay also has the advantage of being specific for this pathogen, and does not pick up morphologically related but non pathogenic cysts such as *Entamoeba dispar*. Finally, the assay can be run on a much wider range of samples, such as biopsies and liver aspirates since it does not rely on the presence of morphologically intact parasites.

Stool samples for the molecular test must not be in any fixative as this may cause false negatives.

Amoebic serology
For such test a **minimum** of 0.5ml of serum is required.

The IFAT (screening titre 1/80) is an essential test in cases of suspected amoebic liver abscess (ALA). Such cases produce high titres of about 1/160-1/320, and the test is positive in over 95% of cases of ALA by the end of the first 14 days. However, it appears to give false positives in some cases of non-amoebic liver disease. Consequently it is necessary to confirm a positive result by the Cellulose Acetate Precipitin test (CAP).

The IFAT also gives very good results in cases of amoeboma. In amoebic colitis the test is positive, often at low titre, in about 75% of cases; in cyst passers it is often negative and in other cases it may be positive because of past infection. The test is therefore not suitable for the investigation of vague abdominal symptoms or as a routine check.

However, in addition to hot stool microscopy, rectal scrapes or biopsies, a negative result should be obtained before the use of steroids or surgery for presumed ulcerative colitis or Crohn's Disease.

A Cellulose Acetate Precipitin test (CAP) will be performed if the IFAT is positive. This test is less sensitive than the IFAT. A positive is confirmatory evidence of an active or recently treated infection. A negative CAP in the presence of a positive IFAT may suggest early infection, a treated case, past infection or occasionally, a false positive IFAT. After treatment the CAP is the first to become negative, sometimes as soon as one month but occasionally after one year.

Babesiosis

A tick borne parasitic infection diagnosed by blood film (*Babesia microti* and *Babesia divergens*) or IFAT (*Babesia microti*). A **minimum** of 2ml of **EDTA anti-coagulated blood** (for microscopy) **and** 0.5ml of **serum** (for serology) is required.

Microscopy of thick and thin blood films is required. Serological testing is available (*Babesia microti* only) on discussion with the laboratory.

Cyclosporiasis

Stool samples may be sent for microscopy. Up to three samples may be necessary due to the intermittent excretion of this parasite.

Malabsorption is a relatively common finding in patients with the recently recognised parasite *Cyclospora cayetanensis*.

Cysticercosis (larval *Taenia solium* infection)

Cysticercosis, caused by the presence of the larval stage (cysticercus) of *Taenia solium* in various organs, especially the CNS, is diagnosed by a variety of methods including imaging and serology. A serological service (EITB Immunoblotting) is provided. A **minimum** of 0.5ml of **serum** is required, CSF testing is also available, please provide as much CSF as you are able to spare.

The cysts may occur in almost any situation but are most likely to draw attention to their presence in the brain or eye. Any patient with 'epilepsy' who has resided overseas should be investigated.

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Intestinal infections with *Taenia solium or saginata* will usually give negative results by serology

Microscopy of stools for ova is recommended in these cases but cannot differentiate to species level.
If sending segments for identification please send without fixative (see below under identification of worms). **HIGH RISK** stickers must be used if *Taenia solium* is suspected

Enterobiasis

An **adhesive tape smear** (Sellotape, Scotch tape (i.e. clear transparent adhesive tape)) taken first thing in the morning from the perianal skin and attached sticky side down to a microscope slide, is the appropriate specimen for detecting *Enterobius vermicularis* ova. While adult worms may be present in stool samples, a negative stool result for worms and ova does not exclude the diagnosis because the ova are laid on the perianal skin.

Fascioliasis

Fascioliasis is caused by *Fasciola hepatica* (the liver fluke of sheep and cattle). Eggs in faeces are often scanty and may not be found in up to 30% of cases. Serology can be helpful and an IFAT test for antibody is available. A **minimum** of 0.5ml of **serum** is required.

Outbreaks of infection with *Fasciola hepatica* (typically following consumption of infected watercress) are uncommon but have occurred in the UK. Occasional sporadic cases are encountered, usually from abroad.

Patients with upper abdominal pain, thought to be hepatic, eosinophilia and fever, should be investigated. Serology is the best method of diagnosis in the early stage of the infection.

The IFAT (screening titre 1/32) has given reliable results. It is species specific. In proven *Fasciola hepatica* infections the titre is in the order of 1/128.

Filariasis

The syndromes produced by the various species of filarial worms are usually associated with eosinophilia. A patient with an eosinophilia who has lived in, or visited, a filaria endemic area might reasonably be tested for filariasis.

The major human filariases are *Wuchereria bancrofti, Onchocerca volvulus, Brugia malayi,* and *Loa loa.*

With the exception of *Onchocerca volvulus*, a definitive diagnosis of filariasis is usually made by the demonstration of microfilariae in the peripheral blood. *Onchocerca volvulus* is diagnosed by demonstration of microfilariae in **skin snips.**

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Twenty millilitres of **anti-coagulated blood (citrate tube)** are required so that the microfilariae can be detected by filtration. Day blood (for *Loa loa*) should be taken between 12noon and 2pm local time and night blood (for *Wuchereria bancrofti*) at 12 midnight. Samples should be kept at room temperature until processed.

	PERIODICITY	COLLECTION TIME(hr/local)
Wuchereria bancrofti	Nocturnal	2400-0200
Brugia malayi	Nocturnal	2400-0200
Loa loa	Diurnal	1200-1400
Mansonella perstans	No periodicity	Anytime
Mansonella ozzardi	No periodicity	Anytime

A filaria ELISA, using *Brugia pahangi* as antigen is used as a “generic” screening test. A **minimum** of 0.5ml of **serum** is required. A negative result does not exclude the diagnosis and this is especially so with onchocerciasis.

The filaria ELISA is a non-specific screening test that is positive in many types of filariasis and in strongyloidiasis. It is most useful in the diagnosis of TPE (Tropical Pulmonary Eosinophilia) where high antifilarial antibody levels are required to make the diagnosis. Positive results are reported at Levels 1 to 9. Levels 1 and 2 are regarded as weak positives; Levels 5 and over are strong positives.

Reactive symptomatic cases with moderate eosinophilia tend to give high level positives. Non-reactive cases, which may be asymptomatic though microfilariae are present, give low levels of positivity and may be negative. Known causes of false positive results are Hookworm (about 50% of cases) and occasionally *Ascaris* infection. We are unable to speciate Filaria infections using our ELISA test. This may be done if microfilaria are seen in a blood film or by staining the microfilaria obtained by filtration.

Free Living Amoeba

A PCR is available upon discussion with the Consultant Parasitologist or SpR for the diagnosis of *Naegleria fowleri, Balamuthia mandrillaris* and Acanthamoeba species. The sample should be CSF or Brain/tissue and received in the laboratory as soon as possible.
Culture is available for *Naegleria fowleri* or *Balamuthia mandrillaris* on discussion with the Consultant Parasitologist or SpR. The sample should be CSF or Brain/tissue and received in the laboratory as soon as possible.

Culture for Acanthamoeba species should be referred direct to the LSHTM.

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Giardiasis

(See also intestinal protozoa)

Giardia trophozoites are only detectable when stools are examined within 4 hours of voiding. *Giardia* cysts are frequently excreted intermittently so that a minimum of **six stools** may be required for microscopic exclusion. *Giardia* can be demonstrated in **duodenal / jejunal juices** if examined within 4 hours.

The molecular test for *Cryptosporidium* species, *Giardia intestinalis* and *Entamoeba histolytica* offers several advantages over standard microscopy based diagnostics. The assay is significantly more sensitive (greater than ten fold improvement in the limit of detection for some species) than light microscopy. In addition the assay is semi quantitative and can therefore reveal detailed information on the response of a patient's parasite load to subsequent drug therapy.

Stool samples for the molecular test must not be in any fixative as this may cause false negatives.

Giardia serology is no longer available, please send an unfixed stool sample for microscopy and PCR.

Hydatid disease

Hydatid ELISA is performed at HTD. The diagnosis should be considered in individuals who have visited an endemic area and have a space-occupying lesion in any organ, especially the liver. The diagnosis of hydatid disease depends upon a compatible clinical picture, serology and imaging.

Serological cross-reactions, giving rise to false positives, can occur with sera from patients with other parasitic infections, notably larval cestodes and filarial worms, and with some neoplasms. False negatives may occur and are more common in the case of non-hepatic hydatid cysts.

A **minimum** of 0.5ml of **serum** is required, CSF testing is available, please provide as much CSF as you are able to spare.

Aspiration of a cyst should be considered only after taking expert advice and, if felt to be indicated, should be conducted in a centre experienced in the management of hydatid disease. If viability is required the aspirate should be kept at room temperature and reach us with 24 hours.

If you have any queries concerning the diagnosis in a suspected case of hydatid disease, please contact us for advice.

Identification of Worms

Tapeworm segments for identification – please send in saline, **DO NOT** send in Formalin or other fixative as this prevents identification beyond genus.

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HIGH RISK stickers must be used if *Taenia solium* is suspected.

Other worms, part or whole, please send as they are or in saline, DO NOT send in formalin or other fixative.

Intestinal Helminthiasis

(Excluding *Enterobius* infections). **Stool samples** should be forwarded with the minimum delay. (A minimum of two separate samples should be examined before a diagnosis is excluded.)

Serology is **NOT** available for Ascaris, Anisakis, Capillaria, Clonorchis, or hookworm infections.

Intestinal Protozoa

(See also Amoebiasis, Giardiasis and Microsporidia).

Stool samples for the demonstration of trophozoites, cysts and oocysts should be forwarded with the minimum of delay.

Microsporidiosis from HIV positive patients
Requests for microsporidiosis should be clearly marked
Intestinal and tissue microsporidia are found almost exclusively in immunocompromised patients. **Stool, tissue and urine samples** may be sent for examination as appropriate.

PCR is the preferred diagnostic tool, please see Microsporidia below

No serology is available at HTD for these parasites

Leishmaniasis

For Diagnosis and Species Determination of Leishmania, please send:

Cutaneous and Mucocutaneous Leishmaniasis
Punch Biopsy; Take from the edge of the lesion. Place in viral transport media containing antibiotics, but not antifungals, and send to Parasitology at HTD.
If histology is required take second biopsy, or cut original biopsy in half vertically through the epidermis and tissue. Put half in viral transport media (as above) for Parasitology at HTD and half in formal saline for histology.

Slit skin smears; Take from the edge of the lesion, onto a slide. Air dry and then fix with methanol.

PCR can be used to detect and speciate *Leishmania* from cutaneous cases. Contact microscopy section of laboratory for advice.

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Visceral
Bone marrow or Splenic aspirate; Please send 2 methanol fixed slides and a **small** amount (less than 1ml) of sample in a sterile EDTA tube (e.g. Vacutainer purple top).

An attempt should always be made to find *Leishmania* from aspirated material (bone marrow or spleen) by microscopy or PCR - contact laboratory for advice.

For all of the above conditions.
Histology Sections; Please send at least 6 normal thickness sections on slides or in a small screw capped tube.

Please do not send samples over the weekend.

Please send a travel history with all specimens.

Serology for Leishmania
For Serology a minimum or 0.5ml of serum is required.

Note: negative serology does not exclude the diagnosis of visceral leishmaniasis, particularly in sera from HIV positive patients.

Serology is **not** helpful in the diagnosis of cutaneous infections.
In mucocutaneous leishmaniasis serology is usually seropositive except in early cases.

A Direct Agglutination Test (DAT) for Leishmaniasis using formalinised promastigotes of *Leishmania donovani* stained with Coomassie blue is the standard serology test and a rapid test (rK39) antibody detection assay is also provided. The DAT is considered positive when the titre exceeds 1600 and in visceral leishmaniasis titres may rise to 51,000 or above. The rK39 test is reported as positive or negative, with no titre available.

Microsporidia

The molecular test for microsporidial species offers several advantages over standard microscopy based diagnostics. The assay is significantly more sensitive (greater than one hundred fold improvement in the limit of detection) than light microscopy. In addition the assay is semi quantitative and can therefore reveal detailed information on the response of a patient's parasitic load to subsequent drug therapy. Finally, the assay can differentiate between morphologically identical microsporidia, a feat only possible previously with electron microscopy. Therefore phenomena such as resistance of *Enterocytozoon bieneusi* to albendazole therapy can be considered in a real time process.

Stool samples for the molecular test must **not** be in any fixative as this may cause false negatives.

Microscopy can still be performed on corneal scrapes.

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Malaria

Suspected malaria is a medical emergency.

The infection is best diagnosed by submitting a **minimum of 2ml of EDTA anti-coagulated blood** with the minimum of delay, so that thick and thin films can be made in the laboratory. A less satisfactory alternative is to submit stained thin blood films plus unstained thick films for examination. Delay in receipt of EDTA sample can adversely affect the integrity of the sample and consequently make accurate diagnosis difficult.

Antigen detection by immunochromatography is available on request.

Malaria serology is **not** suitable for diagnosing current infection.

Malaria (past infection)

Serology for malaria may be requested:
1. If for some reason it is important to attempt a retrospective diagnosis.
2. For the investigation of splenomegaly or nephrotic syndrome in a patient who might have been exposed to malaria.

It is **not** recommended for the investigation of acute fever, as urgent blood film examination is the method of choice. An ELISA assay is performed using *Plasmodium falciparum* and *vivax* antigens. Positive results will be reported as an optical density and a cut-off point will be stated. A **minimum** of 0.5ml of **serum** or EDTA **plasma** is required.

Sera/plasma from suspected Tropical Splenomegaly Syndrome patients will be tested by IFAT if the ELISA is positive.

The malaria ELISA used at HTD cannot be used to speciate malaria infections.

Schistosomiasis

Definitive diagnosis is by demonstration of the characteristic ova in clinical material. For *S. haematobium,* a terminal urine sample (the last 10 to 20ml of urine passed) is required. Faecal samples are the best specimens for the detection of *S. mansoni* (and *S. japonicum*) but as *S. mansoni* and *S. haematobium* overlap in geographical distribution and can affect both genitourinary and alimentary systems a **terminal urine sample and a minimum of three faecal samples** should be sent from all patients being investigated for schistosomiasis when serology is positive. Biopsy material (unfixed) from rectum, sigmoid or bladder is valuable for the detection of ova by crush preparation and permits assessment of their viability. If biopsies are taken, fixed material should also be sent for histology. Rectal / sigmoid scrapings are also useful samples for the diagnosis of schistosomiasis. Such samples must be sent to the laboratory by prior arrangement only.

Serology:
A **minimum** of 0.5ml of **serum** is required.

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The test should be requested on patients known to have been exposed to fresh water in endemic areas. It starts to become positive approximately six weeks after exposure.

Deposition of ova commences at about this time but their first appearance (e.g. in urine) may be delayed for several months. Confirmation of the diagnosis by finding ova should be sought where possible.

The ELISA is reported to detect about 96% of *Schistosoma mansoni* and 92% of *Schistosoma haematobium* infections. The test does not distinguish active from treated infections. The actual time taken to become seronegative post treatment varies, but in some patients the test may remain positive for over two years after treatment.

Positive results are reported at Levels 1 to 6. Levels 1 and 2 are regarded as weak positives; Levels 5 and over are strong positives.

It is known that patients may become seropositive through contact with cercaria from animal species of schistosome and probably when harbouring unisexual infection with human species. The schistosomal egg antigen used in the ELISA may cross-react with the sera of trichinosis cases or with those of hepatitis cases in some instances.

Currently it is not possible to speciate using our serology ELISA test.

Strongyloides

Often associated with mild abdominal symptoms, strongyloidiasis is also an occasional cause of Loeffler's syndrome and, in fulminating cases, may cause secondary bacterial septicaemia or meningitis.

Strongyloides is diagnosed by **faecal microscopy** and stool culture for the demonstration of larvae. The larvae may not be present in every specimen. *Strongyloides* larvae (and adults) can also be demonstrated in **duodenal / jejunal aspirates**. Duodenal string testing is more sensitive.

Faecal specimens should NOT be refrigerated before sending if Strongyloides culture is required.

Serology for strongyloidiasis (ELISA) is available. The test is indicated for the investigation of eosinophilia or if there is a good clinical history to suggest strongyloidiasis.

A **minimum** of 0.5ml of **serum** is required. There is known to be cross reaction between filaria and strongyloides in ELISA tests.

Toxocariasis

Serology is the method of choice for the diagnosis of toxocariasis. The ELISA is usually performed on serum, but can be undertaken on vitreous humour where appropriate. Please contact the serology section if you intend to send vitreous or aqueous fluids.

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A **minimum** of 0.5ml of **serum** is required.

The Toxocara IgG antibody ELISA test against larval excretory/secretory antigen is the most appropriate method for diagnosis. Sensitivity is 91% and specificity is 86% (with cross reactivity possible with strongyloidiasis, trichinosis, amoebiasis and fascioliasis). Results are expressed as an optical density value.

Positive ELISA tests will be confirmed using a Western blot.

A Negative ELISA on SERUM does not exclude ocular toxocariasis. Vitreous sampling may be necessary to exclude ocular toxocariasis.

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Toxoplasmosis

Please refer samples to the Toxoplasma Reference Laboratory (TRL) at Singleton Hospital, Swansea. (General enquiries: 01792 285058)

Trichinosis

Crush preparations of fixed muscle biopsy specimens may reveal larvae. Biopsies should also be fixed and sent for histology. Serology is usually deployed for the diagnosis of this condition. A **minimum** of 0.5ml of serum is required.

Except in the rare event of an outbreak in the UK, serology is usually requested for symptoms suggestive of the stage of muscle encystment: myalgia, eosinophilia, and, in the early stages, fever. The IFAT (screening titre 1/32) has proved reliable and specific with positive titres of about 1/128.

Trypanosomiasis

African trypanosomiasis is caused by *Trypanosome brucei rhodesiense* or *gambiense*. This disease is restricted to Africa. Diagnosis is made by examining stained blood films or by antibody detection. CSF microscopy and serology may occasionally be required in cases with neurological involvement.

American trypanosomiasis (Chagas disease) is caused by *Trypanosoma cruzi*.
Once confined entirely to the Americas it has now spread to other continents.

Diagnosis is made by examining stained blood films (taken within two months of infection the acute phase or reactivation in cases of immunosuppression) or by antibody detection at any time outside these periods.

Trypanosomes disintegrate rapidly on removal from the body, therefore it is vital that specimens for microscopy are examined rapidly. EDTA whole blood must be examined within 24 hrs and CSF within 20 minutes of taking the sample.

A **minimum** of 2ml of EDTA anti-coagulated blood **and** a **minimum** of 0.5ml of serum is required.

CSF testing is available, please provide as much CSF as you are able to spare.

Sera are screened by IFAT for *Trypanosoma brucei.* The usual titre for screening is 1/20.

An ELISA is used for screening and serodiagnosis of *T. cruzi* with IFAT performed on ELISA positive samples.

Please give the relevant travel history so that the appropriate species can be tested for.

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Visceral Larva Migrans

Serology offers almost the only prospect of specific diagnosis. Requests should be made for tests for filariasis, strongyloidiasis and toxocariasis. A **minimum** of 0.5ml of serum is required.

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RETENTION OF SAMPLES

Please note that we do not keep all samples once tested so if extra tests are required please phone the laboratory at the earliest opportunity to request the additions, please see table below for approximate sample retention times:

Serum and CSF supernatant for serology tests	6 months unless specifically requested to be saved (or found to be positive)
Citrated blood for filarial microscopy	Discarded after filtration
EDTA Blood for microscopy	Forty eight hours
Body fluids inc semen sputum Aspirates BAL duodenal and jejunal cyst fluids stool Urine Perianal swab	48 hours after final report produced by Parasitology Laboratory
String test Skin scrapes Skin snips Swabs Rectal scrapes Rectal snips Sellotape slide	Discarded after processing and testing
Biopsies Bone marrow Slit skin smears	6 months (unless all sample used in testing)
Ectoparasites Adult worms	1 year
Tapeworm Segments	48 hours after final report produced by Parasitology Laboratory
Ticks	Not kept (sent to ref lab for further ID)

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REPORTING TIMES FOR LABORATORY INVESTIGATIONS

The reporting time is defined as the period from the receipt and booking in of a specimen to the time the report is ISSUED to the individual requesting the test.

Clinically important requests will be given priority and the results telephoned to you at the earliest opportunity.

A table listing the range of tests for parasitic diseases that are undertaken in the Department of Clinical Parasitology is available as a separate turn around times document. Some tests are restricted to UCLH requests only.

Routine serological analyses are undertaken in batches.

The following serology tests are referred to the Mahidol University, Thailand.

- *Angiostrongylus*
- *Paragonimus*

We would hope for a 28 day turnaround from sending of a sample to another reference laboratory to receiving a result and reporting it on our computer system.

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Section 6

Results and advisory service

For microscopy where possible, we aim to provide a 24-hour turnaround time, within the working week. All microscopy tests can be performed and reported by telephone within 24 hours of receiving a specimen if prior notice is given.

Telephoned results:

Results are telephoned under the following circumstances:

- If it is thought that a result might lead to an immediate change in patient management.
- If further information is required to decide whether the submitted sample should be processed further.
- If a telephoned result has been requested.

Results will usually be telephoned by the Specialist Registrar or by the individual who has performed the test, but if clinical advice is likely to be needed the call may be made by the Consultant Parasitologist or Deputy. If a telephone number, telephone extension or bleep number has been indicated on the report, the call will be made to that number.

Parasite Serology - Normal Reporting Practice

It is most economic to carry out serological tests in batches and, in general, serological tests are not necessarily performed as soon as a specimen is received. Most tests are batched weekly, thus when tests are carried out at the Department of Clinical Parasitology, written reports may not be available for eight days after the specimen has reached the laboratory. See the table published on the same web page as this manual for in laboratory turnaround times.

When several tests are to be carried out on the same specimen then reports are issued as results become available. Reports are not necessarily delayed to allow all tests to be reported at once.

If urgent results are required or if you want to know when a particular result will be available when please contact the laboratory (020 3447 5413).

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Storage of Results

All records are currently maintained in the department for a minimum period of ten years.

Obtaining Results by Telephone

Although written reports are issued as soon as they are available, the laboratory is happy to make results available by telephone when these would be helpful. Users are asked to use this service only when necessary as it does delay the routine work of the laboratory.

UCLH information governance policy:

Wherever possible personal information should not be transferred by FAX. If, for reasons of urgency, it is necessary to use FAX, rather than mail or courier, the FAX should be sent to a designated Safe Haven. Therefore if we receive a request to fax results we require a safe haven fax number, and we will only fax results if a paper copy is required urgently.

Obtaining advice and information

If you need of advice on clinical interpretation of results, Professor P.L. Chiodini or the Specialist Registrar can be reached on 020 3447 5418 or 5809. If the advice relates to a particular result it is helpful if the clinical details and laboratory reference number are available.

For advice on the types of samples and containers appropriate for different tests please contact the Microscopy section on 020 3447 5414, or serology section on 020 3447 5413 or the Laboratory Manager on 020 3447 5411.

If you are unsure which of the above numbers is appropriate please telephone the laboratory general enquires number on 020 3447 5418 and the Department of Clinical Parasitology staff will put you in touch with the appropriate section.

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